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# Genomics in the detection of damage in microbial systems: cell wall stress in yeast

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## Abstract

*Saccharomyces cerevisiae*, like other microorganisms, has evolved different mechanisms to survive under adverse conditions. The adaptation of yeast to cell wall stress is mainly regulated by mitogen-activated protein kinase (MAPK) pathways. The characterization of genome-wide transcriptional profiles to different cell wall stresses has allowed the identification of those genes important for cell wall remodelling under these circumstances. Moreover, profiling of mutant strains deleted in different elements of these pathways revealed the complexity of the signal transduction machinery responsible for regulating adaptation responses to cell wall stress in yeast. In addition to increase understanding of these adaptive responses, the molecular dissection of these signalling networks could impact on the development of effective new antifungal agents.

**Keywords:** Cell integrity, cell wall, genomics, MAPK, stress, transcriptome

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All living organisms, including fungi, use numerous signal transduction pathways to sense the environment, generate adequate cellular responses, and therefore proliferate in a large range of biological niches [1]. There are many stressful conditions in response to which fungal cells have developed sophisticated signalling cascades. In this way, yeast cells can respond to, adapt to and survive many stressful conditions, e.g. osmotic stress, heat, high salt, pH, UV irradiation, oxidative stress, cell wall stress, or stress caused by antifungal compounds. In the yeast *Saccharomyces cerevisiae* (budding yeast), most of these responses are regulated through mitogen-activated protein kinase (MAPK) pathways. This organism has been shown to be an excellent eukaryotic model system with which to extensively study these MAPK signalling pathways. In the budding yeast, four MAPKs—Fus3, Kss1, Hog1, and Slt2/Mpk1—control mating, filamentation/invasion, high-osmolarity, and cell integrity pathways. They are activated in response to mating pheromones, starvation, osmolarity, and cell wall damage, respectively [2].

Yeast cell integrity depends on a particular external envelope, the cell wall, which is a macromolecular complex whose mechanical strength allows cells to support turgor pressure against the plasma membrane [3,4]. Because of the importance of the cell wall for survival, stress conditions that alter this structure lead to the activation of a cellular response named the 'compensatory mechanism' [5] in an attempt by the cell to survive. This response is characterized by: (i) an increase in $\beta$-glucan and chitin contents; (ii) changes in the relationship between cell wall polysaccharides; (iii) increases in the amounts of several cell wall proteins; and (iv) the re-localization of important proteins from the cell wall construction machinery to the lateral cell wall.

DNA microarray technology provides a powerful tool for genome-wide transcriptional profile characterization, therefore contributing to our understanding of the molecular basis of stress adaptation responses (Fig. 1). The transcriptional programme of the yeast in response to both constitutive (mutants deleted in genes important for cell wall biogenesis) and transient (presence of cell wall-perturbing agents such as Congo red, zymolyase or pneumocandins) cell wall damage conditions has been extensively studied by means of DNA microarray experiments [6–9]. Analysis of the transcriptional responses to the three different cell wall-interfering compounds named above—Congo red (a compound that binds to chitin, interfering with proper cell wall construction), zymolyase (which affects cell wall integrity as a result of the presence of a main $\beta$-1,3-glucanase activity and a residual protease activity), and pneumocandins (inhibitors of $\beta$-1,3-glucan synthase)—reveal that the main functional groups of upregulated genes are those involved in cell wall biogenesis, metabolism and generation of energy, signalling, and genes of unknown function. Comparison of the different responses

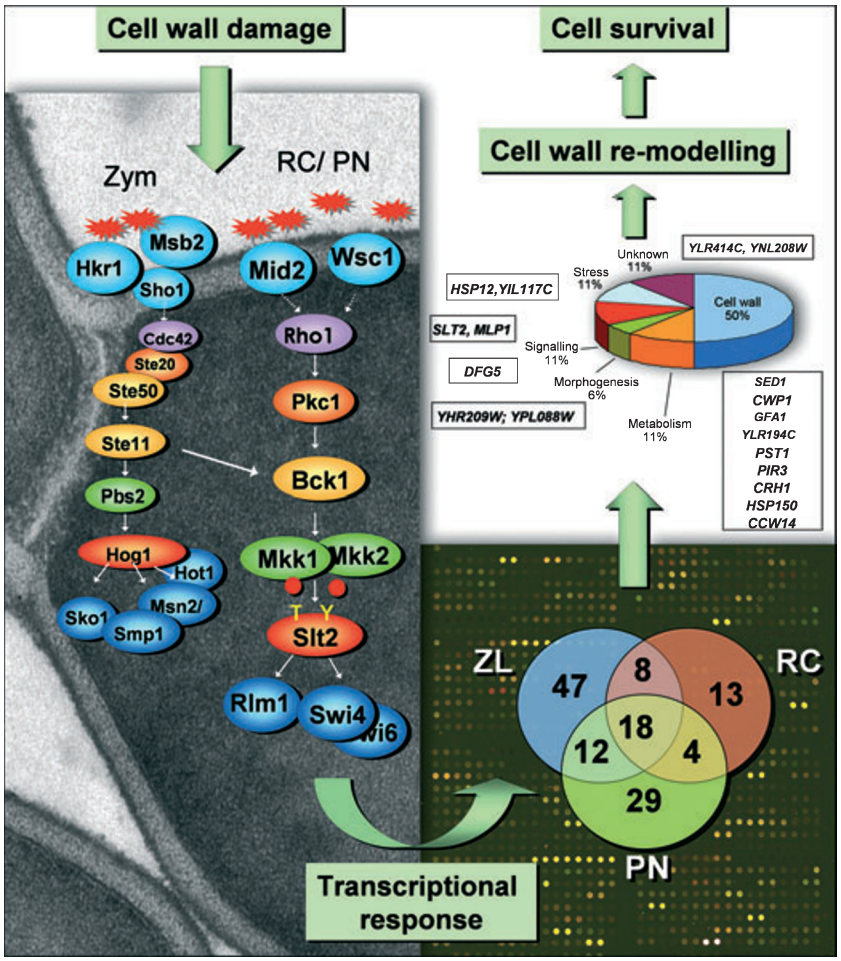


**Fig. 1.** Yeast cellular responses to cell wall stress. Yeast cells respond to cell wall damage caused by different cell wall stresses (ZL, zymolyase; RC, Congo red; PN, pneumocandin) by activating a transcriptional programme that includes the upregulation of different sets of genes. A common signature of 18 genes, most of them related to cell wall biogenesis, is induced in all these situations. As a consequence of this cellular adaptation response, the cell wall is remodelled for survival. These transcriptional responses are mainly regulated by the CWI pathway, but the HOG pathway is also involved in regulation of responses to stress mediated by zymolyase.

reveals not only the existence of specific transcriptional adaptation profiles for each situation, but also the presence of a common signature of 18 genes that is induced in all these situations (Fig. 1). Interestingly, 50% of this response includes genes directly involved in cell wall remodelling (*SED1*, *CWP1*, *GFA1*, *YLR194C*, *PST1*, *PIR3*, *CRH1*, *HSP150* and *CCW14*). One of the upregulated genes, *CRH1*, codes for a putative transglycosidase activity that, together with the product of the homologous *CRH2*, is involved in the cross-linking between chitin and $\beta$-1,6-glucan [10]. Transcriptional activation of *CRH1*, together with the re-localization of the protein encoded by this gene to the lateral cell wall, is part of the mechanism of adaptation to heat stress.

The CWI pathway is the main signalling pathway involved in the regulation of cell wall stress responses. The MAPK of this pathway, Slt2, is encoded by one of the genes transcriptionally induced in the compensatory response. This pathway is activated through several cell membrane proteins (Mid2, Wsc1-4, and Mtl1), with Mid2 and Wsc1 being the main sensors of the pathway. Upon cell wall damage conditions, these sensors interact with the GEF Rom2, activating the small GTPase Rho1, which then interacts with and activates Pkc1 [4]. The main role of activated Pkc1 is to trigger a MAPK module. Phosphorylation of the MAPK kinase Bck1p by Pkc1 activates a pair of redundant MAPKs (Mkk1 and Mkk2), which finally phosphorylate the MAPK Slt2. The phosphorylated form of this protein acts mainly on two transcription factors: the MADS-box transcription factor Rlm1, and SBF. SBF is a heterodimeric complex of two proteins—Swi4 and Swi6—that is involved in the regulation of gene expression at the $G_1$/S transition. As deduced from genome-wide transcriptional studies, activation of the majority of the genes induced in response to Congo red [7], heat shock [11] and zymolyase (R. García and J. Arroyo, unpublished data) is dependent on Rlm1.

Although the cell integrity pathway is the main pathway for cell wall remodelling under cell wall stress, there is also evidence suggesting that different mechanisms are involved in the activation of the MAPK Slt2 under different cell wall stress conditions. Recent data show that the HOG pathway is not only necessary for survival under hyperosmotic conditions but is also involved in adaptation to cell wall stress.

Although cell wall-interfering compounds such as Congo red and zymolyase elicit common genome-wide transcriptional responses related to the set of genes involved in cell remodelling and signalling [7], adaptation responses to these two cell wall stresses are differentially regulated. The response to Congo red depends only on the CWI pathway, whereas the Sho1 branch of the HOG pathway, in addition to the CWI pathway, is also responsible for remodelling the cell wall in response to specific cell wall damage to the $\beta$-1,3-glucan network caused by zymolyase. Zymolyase activates both MAPKs, and Slt2 activation depends on the Sho1 branch of the HOG pathway under these conditions. Thus, sequential activation of two MAPKs (HOG and SLT2) pathways is required for cellular adaptation to this specific cell wall stress [12]. Interestingly, zymolyase cell wall stress is not sensed through sensors of the CWI pathway but depends on the mucin-like proteins Hkr1 and Msb2, recently described as putative sensors of the Sho1 branch of the HOG pathway [13]. Furthermore, Congo red and pneumocandins activate the CWI pathway, but the sensors Mid2 and Wsc1 of this pathway seem to play different roles in this activation. Whereas activation by Congo red of Slt2 and the induction of the transcriptional programme by this drug are mainly dependent on Mid2, the adaptation response to pneumocandins is mainly sensed through the protein Wsc1 (C. Bermejo and J. Arroyo, unpublished data). Thus, differential regulation of cell wall stress responses seems to be a consequence, at least in part, of how cells are able to sense different types of stress.

An interesting aspect of studying all these mechanisms of fungal adaptation to cell wall stress is the possibility of interfering with them. $\beta$-1,3-glucan synthase inhibitors are among the most interesting antifungal compounds recently developed that are applicable to the management of fungal infections. Combined therapies involving the targeting of cell wall biogenesis by inhibiting $\beta$-1,3-glucan synthesis together with drugs inhibiting the mechanisms of adaptation to the antifungal drug can be envisioned as a strategy for the development of future successful antifungal therapies.

## Transparency Declaration

All authors declare no conflicts of interests.

## References

1. Bahn YS, Xue C, Idnurm A, Rutherford JC, Heitman J, Cardenas ME. Sensing the environment: lessons from fungi. *Nat Rev Microbiol* 2007; 5: 57–69.
2. Qi M, Elion EA. MAP kinase pathways. *J Cell Sci* 2005; 118: 3569–3572.
3. Lesage G, Bussey H. Cell wall assembly in *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev* 2006; 70: 317–343.
4. Levin DE. Cell wall integrity signaling in *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev* 2005; 69: 262–291.
5. Popolo L, Gualtieri T, Ragni E. The yeast cell-wall salvage pathway. *Med Mycol* 2001; 39 (suppl 1): 111–121.
6. Boorsma A, de Nobel H, ter Riet B *et al.* Characterization of the transcriptional response to cell wall stress in *Saccharomyces cerevisiae*. *Yeast* 2004; 21: 413–427.
7. García R, Bermejo C, Grau C *et al.* The global transcriptional response to transient cell wall damage in *Saccharomyces cerevisiae* and its regulation by the cell integrity signaling pathway. *J Biol Chem* 2004; 279: 15183–15195.
8. Lagorce A, Hauser NC, Labourdette D *et al.* Genome-wide analysis of the response to cell wall mutations in the yeast *Saccharomyces cerevisiae*. *J Biol Chem* 2003; 278: 20345–20357.
9. Rodriguez-Peña JM, Perez-Diaz RM, Alvarez S *et al.* The 'yeast cell wall chip'—a tool to analyse the regulation of cell wall biogenesis in *Saccharomyces cerevisiae*. *Microbiology* 2005; 151: 2241–2249.
10. Cabib E, Blanco N, Grau C, Rodriguez-Pena JM, Arroyo J. Crh1p and Crh2p are required for the cross-linking of chitin to beta(1-6)glucan in the *Saccharomyces cerevisiae* cell wall. *Mol Microbiol* 2007; 63: 921–935.
11. Jung US, Levin DE. Genome-wide analysis of gene expression regulated by the yeast cell wall integrity signalling pathway. *Mol Microbiol* 1999; 34: 1049–1057.
12. Bermejo C, Rodriguez E, Garcia R *et al.* The sequential activation of the yeast HOG and SLT2 pathways is required for cell survival to cell wall stress. *Mol Biol Cell* 2008; 19: 1113–1124.
13. Tatebayashi K, Tanaka K, Yang HY *et al.* Transmembrane mucins Hkr1 and Msb2 are putative osmosensors in the SHO1 branch of yeast HOG pathway. *EMBO J* 2007; 26: 3521–3533.